STUDIES ON THE GROWTH OF ROOT HAIRS IN SOLUTIONS

IX. The pH-Molar Rate Relation for Collards in Calcium Nitrate

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A preliminary study of the growth of root hairs of collards in calcium nitrate, reported in No. II of this series,[1] represented an attempt to analyze the effect produced by increasing the concentration of the salt in the culture solution. The two separable factors in evidence were the chemical effect of the salt and the osmotic effect of the solution. In a solution containing 0.003 *M* sucrose, in addition to the optimum concentration of calcium nitrate, there was the same amount of retardation in growth that was obtained through the addition of an equimolar concentration of calcium nitrate. If, however, these additions were made to sub-optimal concentrations of calcium nitrate, there was a decrease in the percentage of retardation due to sucrose and a positive acceleration upon the addition of equimolar amounts of nitrate. It seemed probable therefore that the effect of osmotic pressure alone was retardation, but that in this latter instance it had been overcome by the stimulative property of the calcium nitrate up to a certain concentration.

[1] Farr, C. H. Studies on the growth of root hairs in solutions.

I. The problem, previous work, and procedure. Am. Jour. Bot. **14**: 446–456. *f. 1.* 1927.

II. The effects of concentration of calcium nitrate. *Ibid.* 497–515. *f. 2.* 1927.

III. The effects of concentrations of $CaCl_2$ and $Ca(OH)_2$. *Ibid.* 553-564. *f. 3-4.* 1927.

IV. The pH-molar-rate relation for collards in calcium chlorid. *Ibid.* **15**: 6–31. *f. 5-10.* 1928.

V. Root hair elongation as an index of root development. *Ibid.* 103–113. *f. 11-15.* 1928.

VI. Structural responses to toxic pH and molar concentrations of calcium chlorid. *Ibid.* 171–178. *pl. 7-9.* 1928.

VII. Further investigations on collards in calcium hydroxide. Torr. Bot. Club Bul. **55**: 223–245. *f. 16-18.* 1928.

VIII. Structural and intracellular features of collards in calcium nitrate. *Ibid.* **55**: 529–553. 1928.

It was suggested by the author that the critical concentration represented by the optimum of the graph might be due to a limiting factor with respect to the amount of calcium which could be absorbed from the solution, modified by the negative effect of the osmotic pressure. The nitrate ion was mentioned as a possible controlling agent in the determination of the amount of calcium absorbed. The addition of calcium nitrate above the optimal concentration could not, then, increase the rate of growth, and the retardative effect of osmotic pressure was shown in the downward slope of the curve. The sharp break in the curve in the region of optimum concentration marked the point where the accelerative effect of calcium nitrate ended and the retardative effect of osmotic pressure began. The gradual slope of the curve in solutions of still higher osmotic pressure was thought to be due to the gradual adjustment of the root hairs to the solutions.

Because of the peculiar relationship of calcium to the growth produced in solutions of varying acidity or alkalinity, it became necessary to determine the pH of every solution used. The present study represents a more complete observation of the behavior of the root hairs in different concentrations of calcium nitrate in hydrogen-ion concentrations varying from 3.5 to 12.0.

The experiments were carried on at the Marine Biological Laboratory, Woods Hole, Mass. The methods and procedure were essentially the same as those described in paper No. IV, of the series noted above. In this present study, however, buffer solutions were obtained which would cover more completely the upper part of the pH range in order to increase the accuracy of the colorimetric determinations from pH 9.5 to pH 12.0. To the series of indicators previously used there were added Brom Cresol Green, Nitro Yellow (pH 10.0–11.6), and Sulpho Orange (pH 11.0–12.6). The entire series of standard solutions with the respective indicators were placed upon a rotating table in order to increase the facility and rapidity with which the colorimetric determinations could be made. Pyrex glass tubing, stop-cocks, and separatory funnels were used as well as the Pyrex flasks for the solutions. A filter pump was employed for aeration instead of the aspirator bottles used formerly. Solutions of the desired concentration of calcium nitrate were prepared from a 0.5 *M* stock solution, and the acidity or alkalinity was adjusted to the desired point by the addition of either nitric acid or calcium hydroxide.

pH	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5
0.100			13.4						18.4					1.1		
0.060		16.6			26.6		25.6				25.9				8.6	
0.030			11.0		34.0		51.3		51.5		30.0	27.3		32.1	28.4	0.0
0.020		12.3			17.2		39.7		62.2		31.6		47.0		25.8	
0.012	0.0		56.2		54.9		72.5		53.9		27.6		54.7	41.6	7.9	
0.008		15.5			64.4		54.7		76.1		57.8		26.3	21.8		
0.004			14.1		53.2		54.2	72.1	64.1		59.2		61.3		49.5	0.0
0.000							0.0	51.0	62.9	39.6	45.2	36.9	85.6		37.4	4.5
Mols.																

TABLE I

Average root-hair elongation in calcium nitrate.

In table I is given a summary of the average rate of root-hair elongation in concentrations of calcium nitrate varying from 0.004 *M* to 0.100 *M* and covering a pH range of 4.0–11.5. This same data is presented in more graphic form in fig. 1. A com-

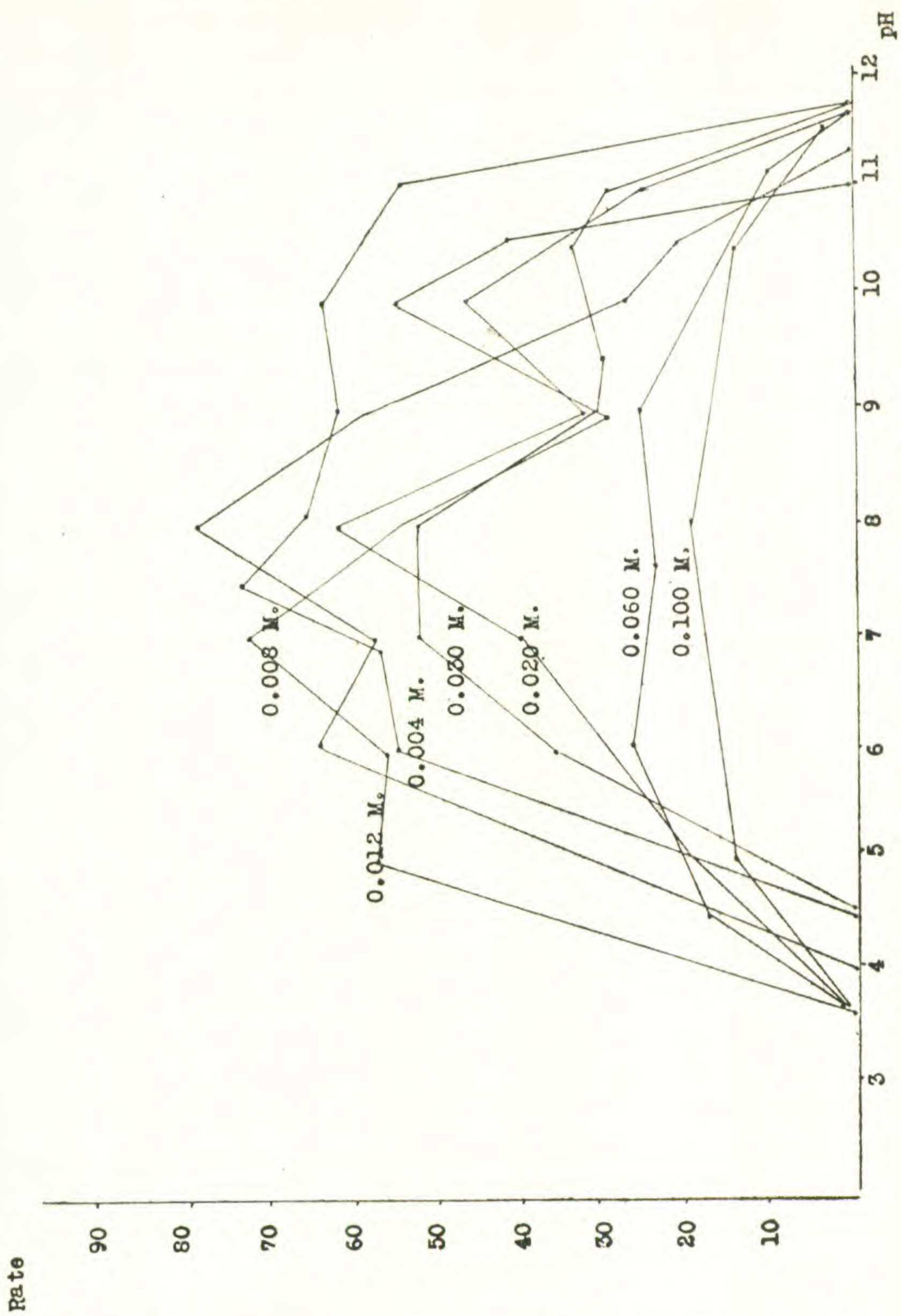

Fig. 1. Average rate of root-hair elongation in calcium nitrate.
C. H. Farr, 1927.

parison of these results with those obtained in solutions of calcium chloride under similar conditions shows a very close agreement. The pH range covered is essentially the same. All curves for the average rate of root-hair elongation in calcium chloride were bimodal with the exception of those in the higher concentrations. These were monomodal in form. In calcium nitrate there are three types of curves, the trimodal in lower concentrations, the bimodal in median concentrations, and the monomodal in higher concentrations. Increase in the concentration of the salt brought about a decrease in the range of acidity and alkalinity which would support growth in both calcium chloride and calcium nitrate. The highest average rate of growth in calcium chloride occurred at a concentration of 0.008 *M* and at a pH of 7.9. The highest average rate of growth in calcium nitrate occurred at a concentration of 0.008 *M* and at a pH of 8.0. The numerical value of this highest average rate in calcium chloride is 88.4, in calcium nitrate, 76.1. This tendency in all solutions of calcium chloride to support a slightly higher rate of growth than that which took place in equimolar solutions of calcium nitrate is obvious. The difference is so small, however, that it may be of no significance. It will be discussed in another connection later.

The maximum rates of root-hair elongation are summarized in table II. These values are obtained from the highest rate of growth attained by any root hair at the pH indicated in the solutions of various concentrations. In calcium nitrate the most rapid growth occurred at a concentration of 0.012 *M* at a pH of 7.0. Similar data in solutions of calcium chloride show the most rapid growth of any individual hair to have taken place at a concentration of 0.020 *M* and at a pH of 6.9.

Many considerations in connection with these curves become clearer when they are presented in terms of three dimensions (fig. 2). This model is based upon fig. 1 and the curve for growth rate in calcium hydroxide. The median or neutral optimum found in calcium nitrate is shown here to be slightly zig-zag. It may be assumed, however, that if the data had been obtained at a pH of 7.5 instead of at 7.0 and 8.0 in the median concentrations that this would have been a straight line also.

The idea advanced in previous papers of the series that the rate of elongation of the root hairs represents an accurate index of the development of the root in general is further substantiated by the findings in solutions of calcium nitrate. Four factors may be compared with respect to the influence of the salt and the pH of the solution, at each of the concentrations used. In a low concentration of calcium nitrate (0.004 *M*) a fairly close correspondence is found between root and root-hair elongation (fig. 3). The upper curve represents the maximum rate of root-hair elon-

pH	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5
0.100			26.2						28.1					2.2		
0.060		35.8			42.0		37.4				39.5				20.9	
0.030			34.3		59.1		69.3		90.1		58.4	56.0		46.7	46.7	0.0
0.020		38.9			46.0		59.7		93.2		63.1		81.4		50.7	
0.012	0.0		71.8		71.5		94.7		77.7		58.1		71.5	65.3	16.6	
0.008		33.3			75.8		78.6		89.4		84.8		68.4	57.5		
0.004			41.1		69.3		67.1	83.9	83.9		80.8		87.9		76.4	0.0
0.000							0.0	62.8	80.1	70.5	55.0	66.2	114.1		57.5	14.1
Mols.																

TABLE II

Maximum root-hair elongation in calcium nitrate.

gation, the next the average rate of root-hair elongation, the third is based upon root elongation, and the last upon the maximum length of root hairs. Root elongation here gives a trimodal

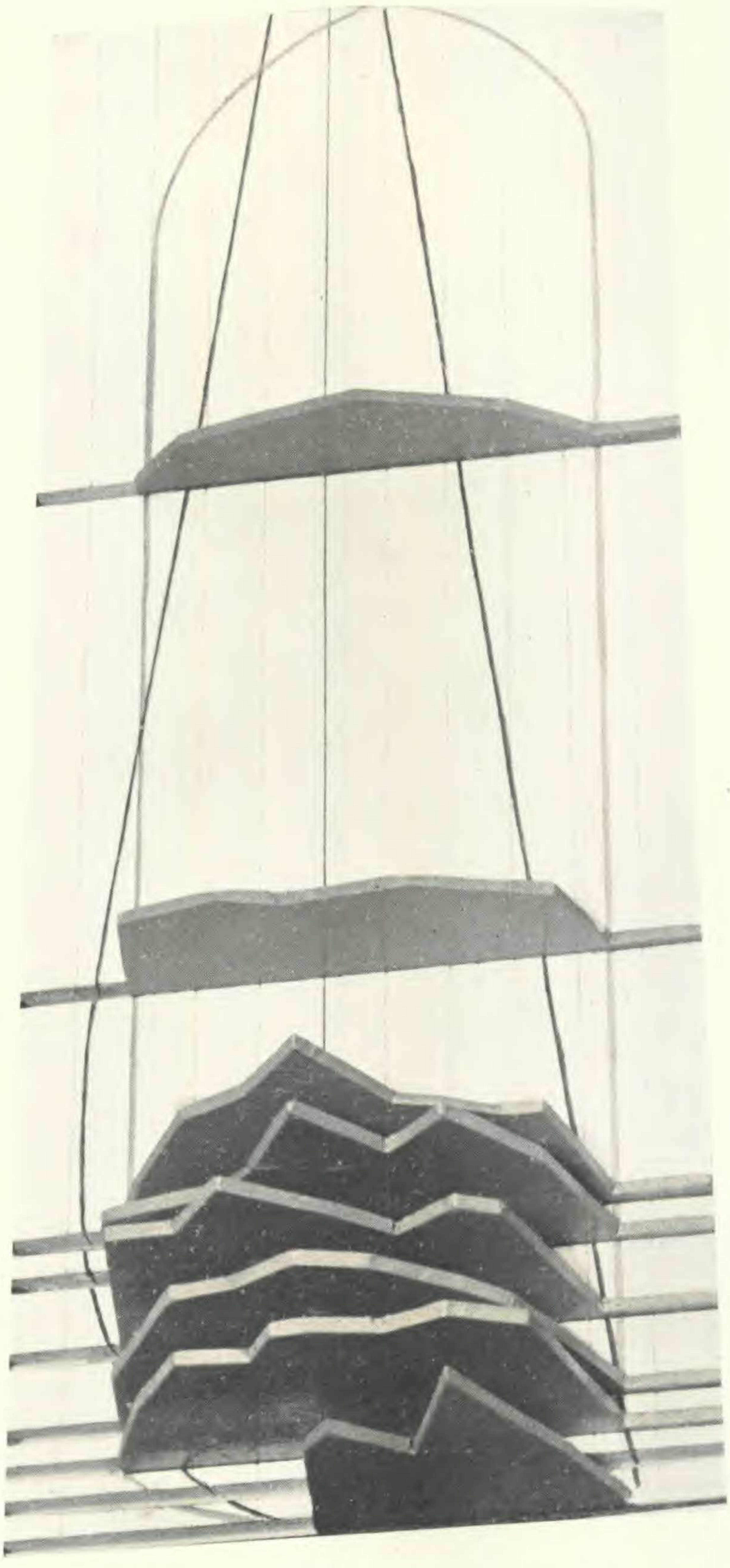

Fig. 2. Three-dimensional graph of the maximum rate of growth of root hairs of collards in calcium nitrate. Vertical parallel lines indicate pH units. Horizontal graphs refer to respective molar concentrations. Uprights represent rate of root-hair elongation. The median vertical line indicates the approximate location of neutrality. The area to the left of this line represents the acid range and the area to the right the alkaline range. The surface bounded by the broad vertical lines indicates the approximate acid and alkaline limits of growth in calcium nitrate. The surface bounded by the converging dark lines represents these same limits in calcium chloride.

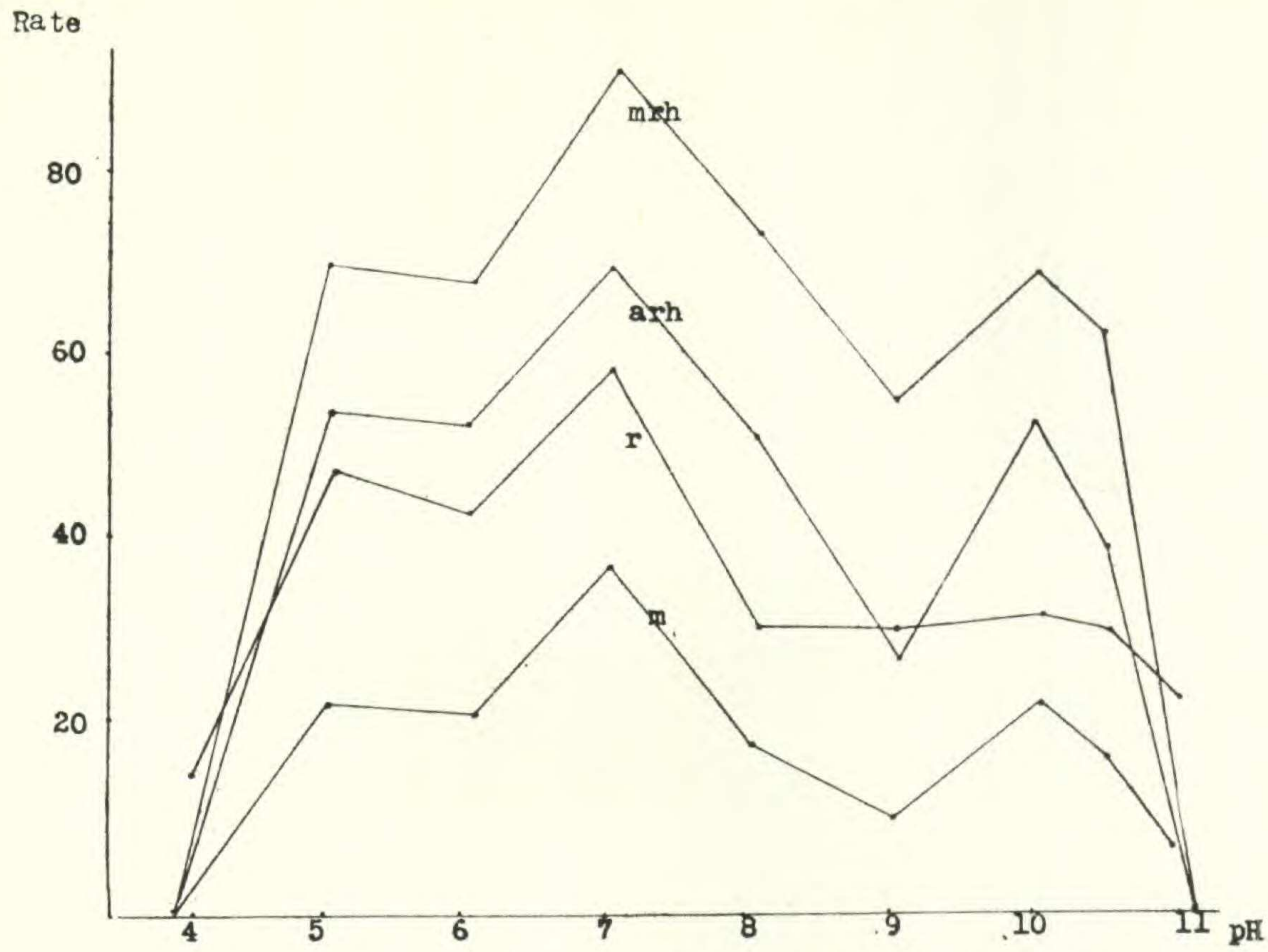

Fig. 3. Root (r) and root-hair elongation in 0.004 *M* calcium nitrate.
C. H. Farr, 1927.

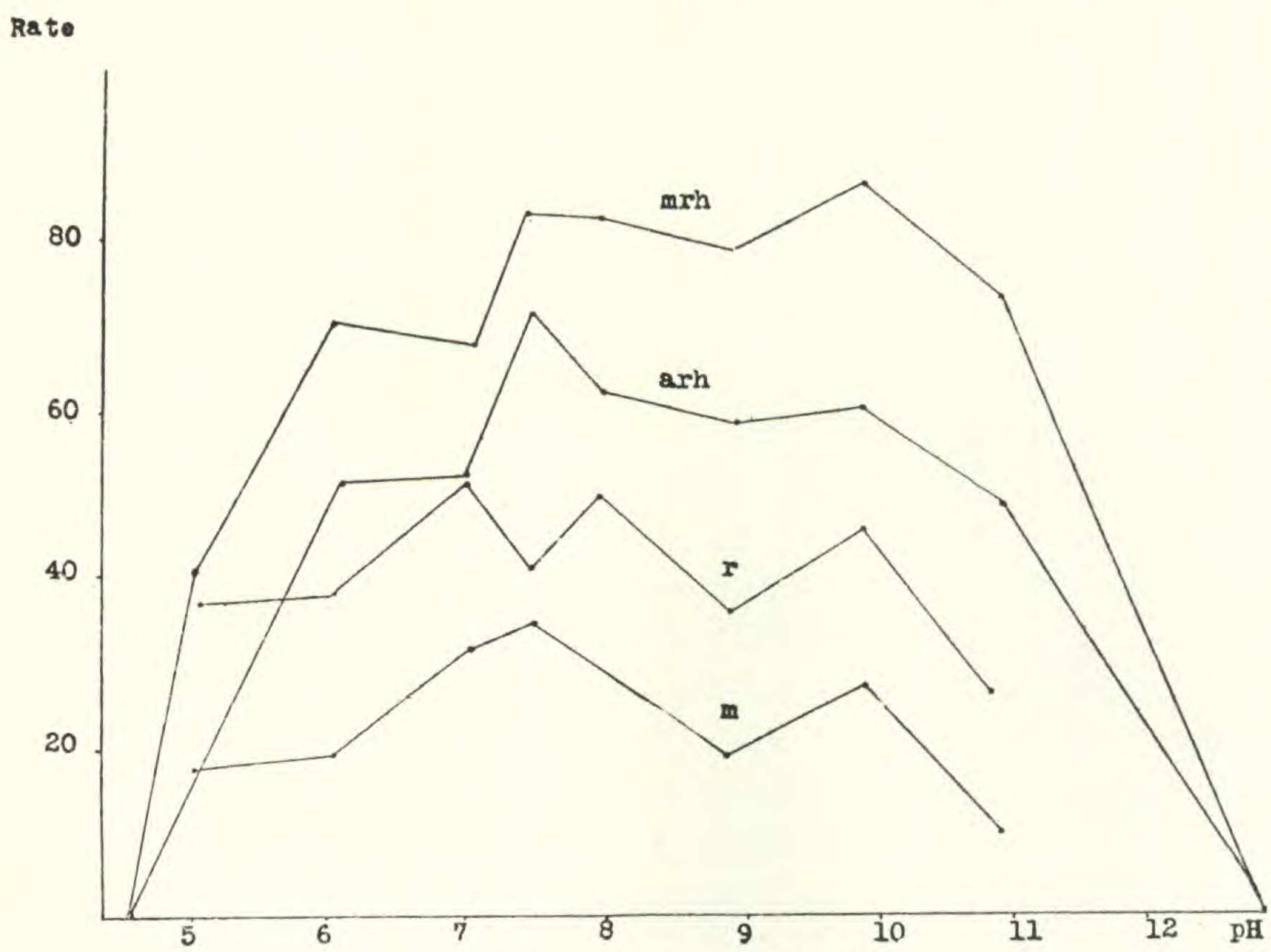

Fig. 4. Root (r) and root-hair elongation in 0.012 *M* calcium nitrate.
C. H. Farr, 1927.

curve such as was obtained for root-hair elongation. The locations of the modes do not, however, correspond except in the alkaline optima. The curve for maximum length of root hairs is bimodal at this concentration.

A concentration of 0.012 *M* calcium nitrate gives more consistent results (fig. 4). The four graphs are almost identical. The root, however, does not give as good differentiation in the alkaline solutions.

In fig. 5, based upon the results in a solution of 0.020 *M* calcium nitrate, there is again a close similarity in the four graphs.

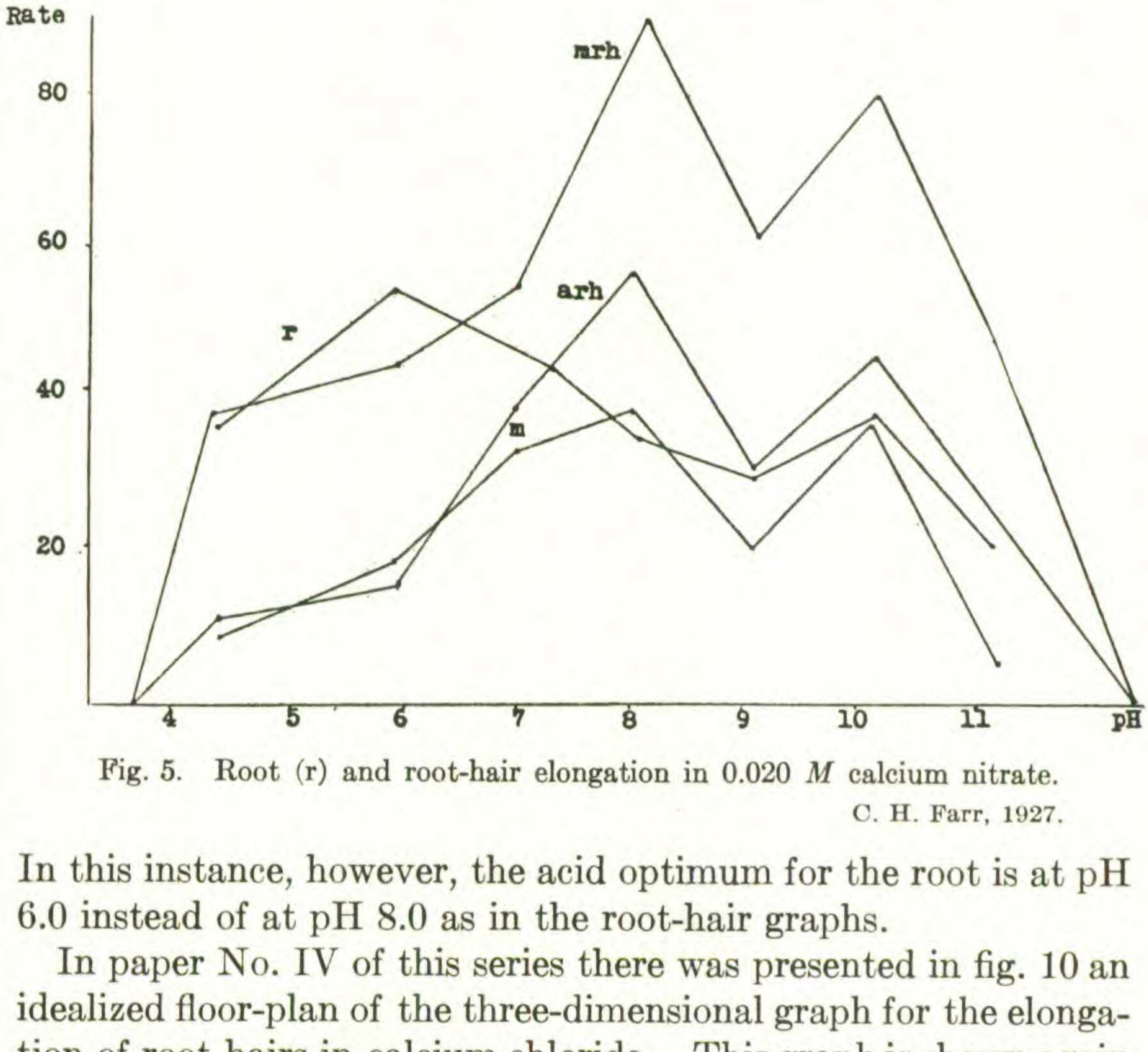

Fig. 5. Root (r) and root-hair elongation in 0.020 *M* calcium nitrate.
C. H. Farr, 1927.

In this instance, however, the acid optimum for the root is at pH 6.0 instead of at pH 8.0 as in the root-hair graphs.

In paper No. IV of this series there was presented in fig. 10 an idealized floor-plan of the three-dimensional graph for the elongation of root hairs in calcium chloride. This graph is shown again in the present study (fig. 6) for the purpose of comparison with the results in calcium nitrate (fig. 7). The median vertical lines in these floor-plans indicate the approximate locations of neutrality. The boundary lines on the right-hand sides represent the alkaline limits of root-hair elongation. The boundary lines

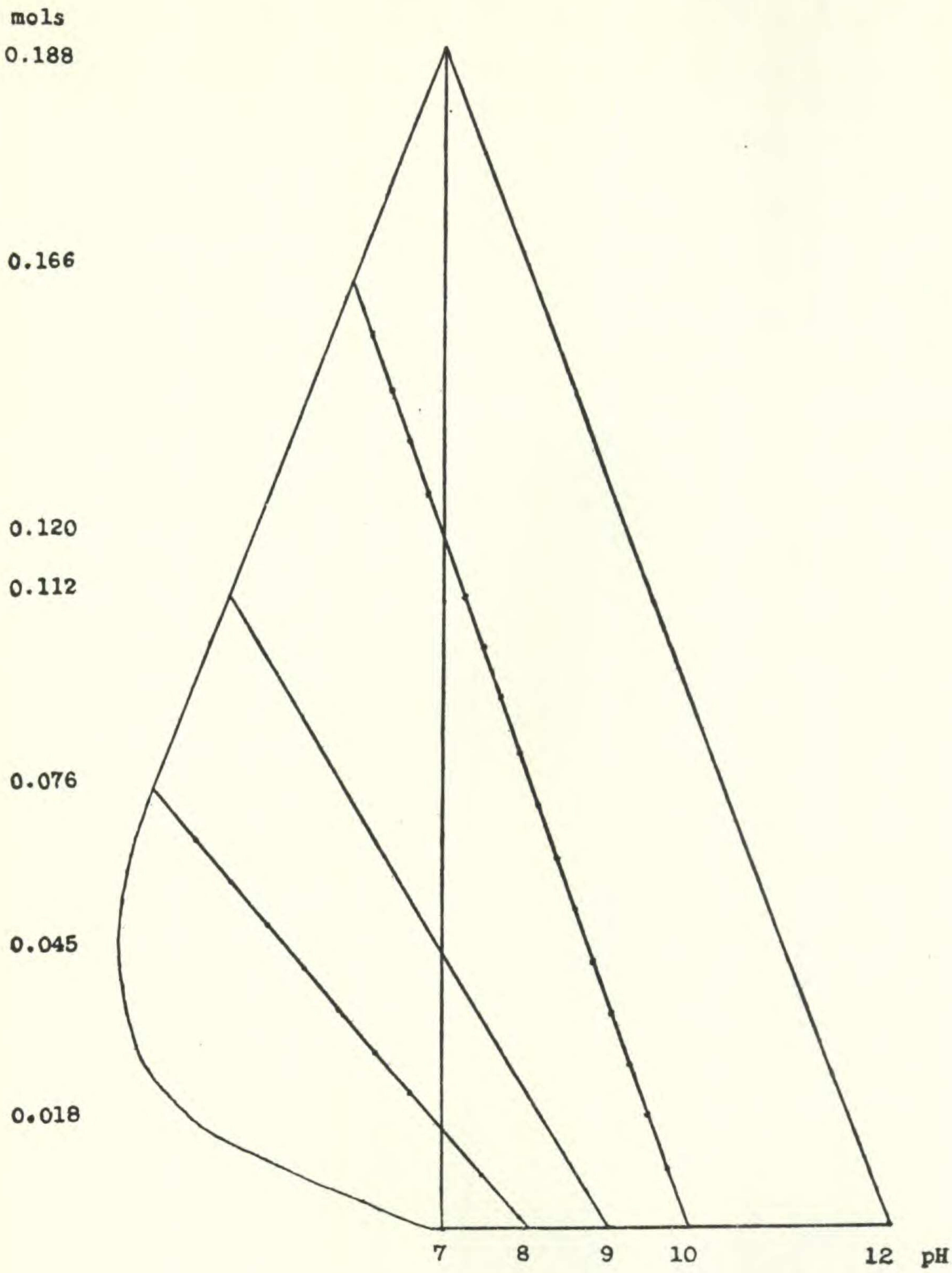

FIG. 6. Tolerance map for collards in calcium chloride solutions of different molar and pH concentrations, based on rate of root-hair elongation. Dotted lines are optima.

on the left-hand side represent the acid limits of root-hair elongation. The oblique lines near to the alkaline limits represent

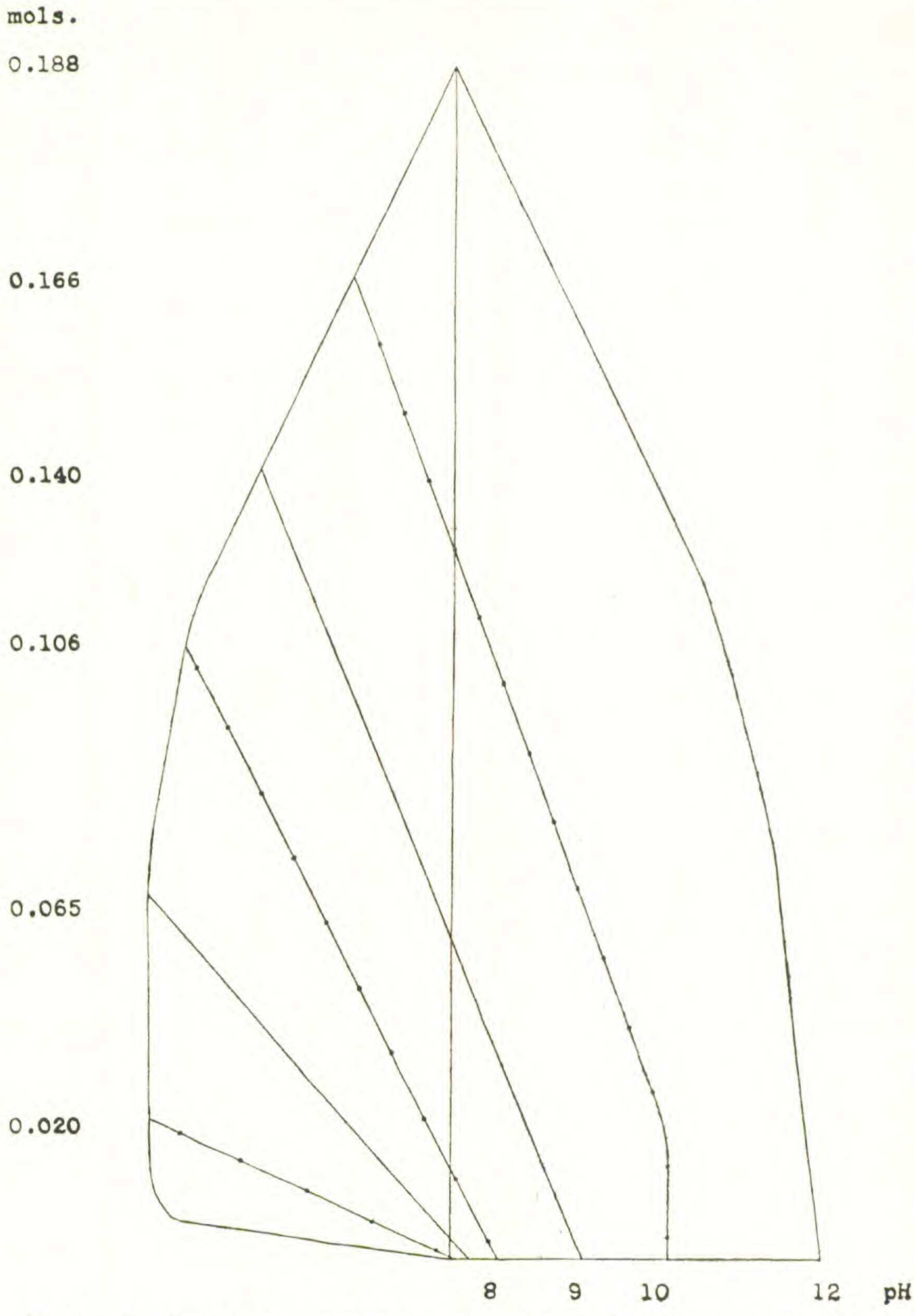

Fig. 7. Idealized floor-plan of tri-dimensional graph for calcium nitrate. Dotted line (——•——•——) indicates maxima.

the alkaline optima. The oblique lines near to the acid limits represent the acid optima. In the graph for calcium chloride the line between the acid and alkaline optima represents the median minimum. The trimodal graph for calcium nitrate has also a neutral optimum with two minimal regions, the one on the acid and the other on the alkaline side of this central modal location. In both instances the floor-plans are seen to take the general form of an isosceles triangle, with the weak acid corner truncated and curved. It has been pointed out previously that root hairs will not grow in the absence of calcium, hence no growth would be expected in hydrochloric acid in the region represented by the projection of the base line to the left of pH 7.0. It has been known also, from various sources, that calcium antagonizes the injurious effects of the hydrogen ion. These floor-plans clearly show that very dilute calcium solutions which are only slightly acid will not permit root-hair growth. One may observe that appreciable concentrations, 0.018 *M* in the case of calcium chloride and 0.020 *M* in the calcium nitrate, will not permit the production of root hairs in solutions of pH 3.5. A graphic representation is given of the fact that the addition of lime to an acid solution or to a soil low in calcium will alter the solution so that it will support optimum growth conditions, since it changes the pH toward or to neutrality and at the same time raises the calcium content.

Some interesting relationships are brought out by superimposing the floor-plan for calcium nitrate upon that for calcium chloride, (fig. 8). Here it is seen that at most concentrations the plant will produce root hairs in a more alkaline solution of nitrate than of chloride. In weak solutions nitrate will support root-hair growth better than will chloride on the acid side of neutrality. In moderate concentrations root hairs will grow in more acid solutions of chloride than of nitrate. It is shown also that the optima of the two salts bear a definite relation to each other, and that in the nitrate, the shifting of both the acid and the alkaline optimum toward the alkaline side and the pushing of the acid limit toward lower acid concentrations make possible the insertion of a new acid optimum.

The differences which have been pointed out between the results obtained in calcium chloride and in calcium nitrate are, however, very slight and may be without significance. The effects pro-

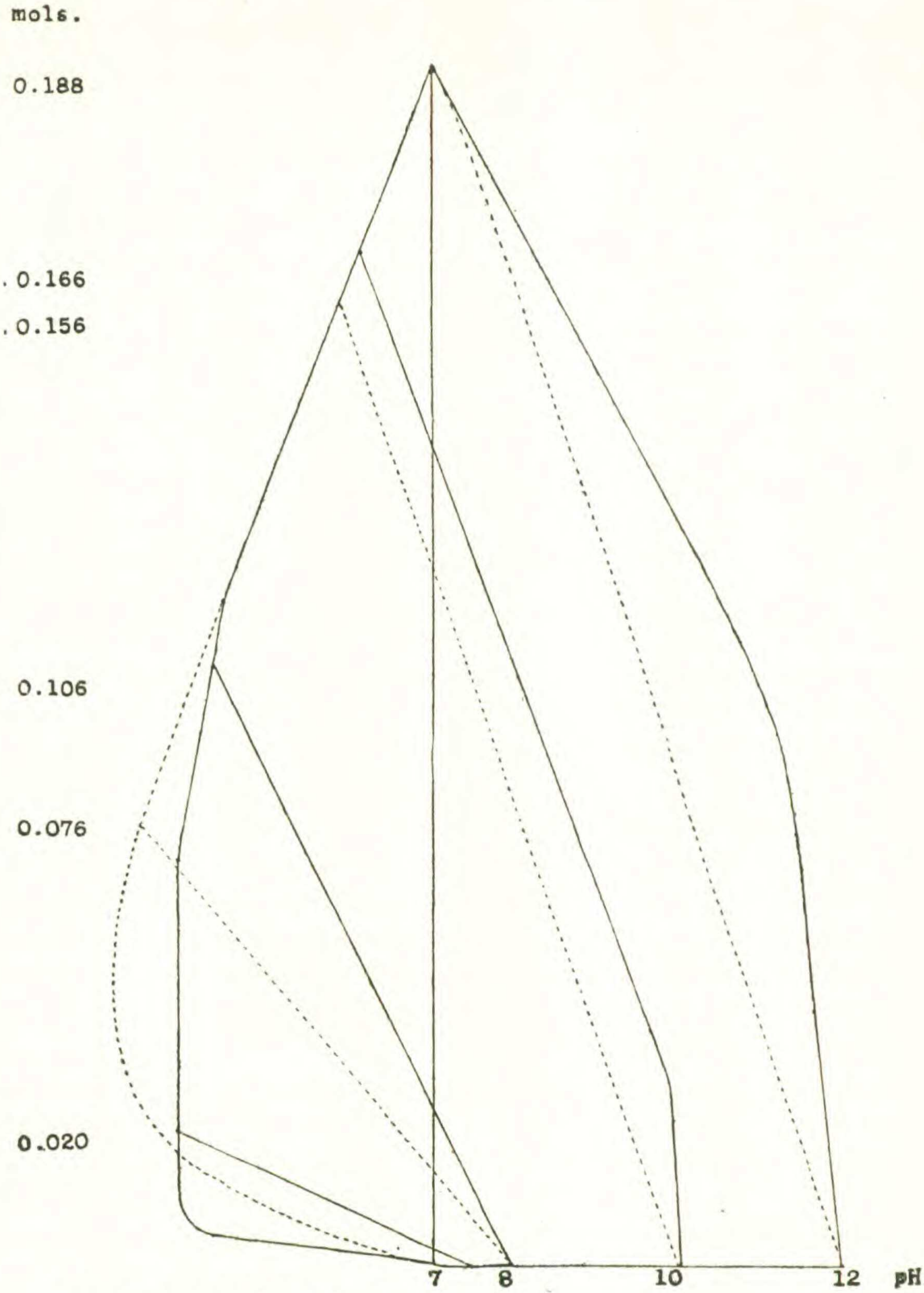

Fig. 8. Floor-plan for calcium nitrate (——) superimposed on calcium chloride (- - - -).

duced by the anions in the two instances are probably more striking because of their similarities than because of their differences.

Further analysis of the entire mass of data obtained for calcium hydroxide, calcium chloride, and calcium nitrate with respect to the possible effects of the anions concerned brings out an interesting correlation. A comparison of the data for the maximum rate of root-hair elongation may be seen in the following table:

Salt	Opt. molar conc.	pH	Max. rate elong.
$Ca(OH)_2$	0.000045	10.0	114.1 microns per hour
$CaCl_2$	0.020	6.9	109.8 microns per hour
$Ca(NO_3)_2$	0.012	7.0	94.7 microns per hour

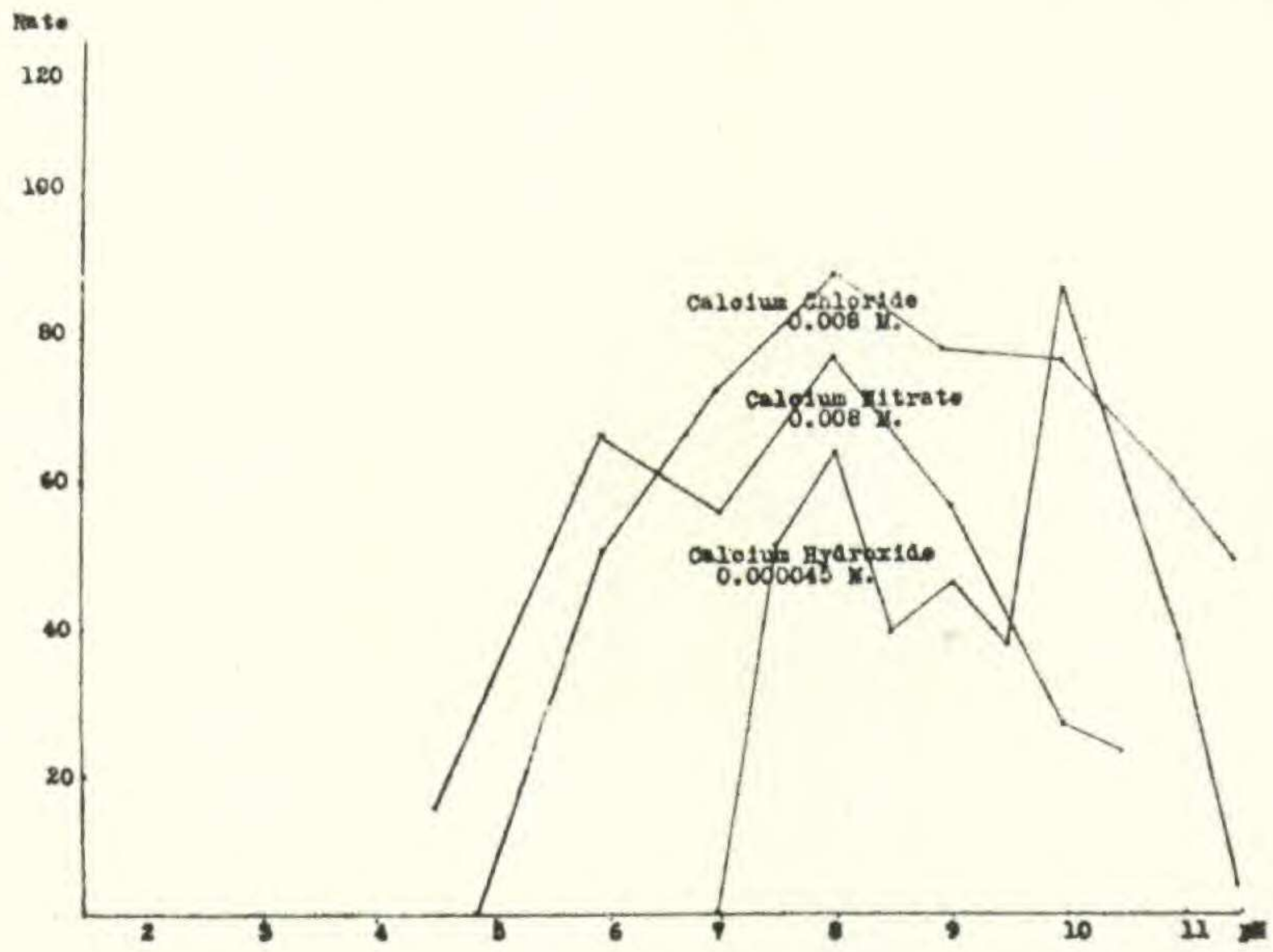

Fig. 9A. Average root-hair elongation in calcium hydroxide, calcium chloride, and calcium nitrate.

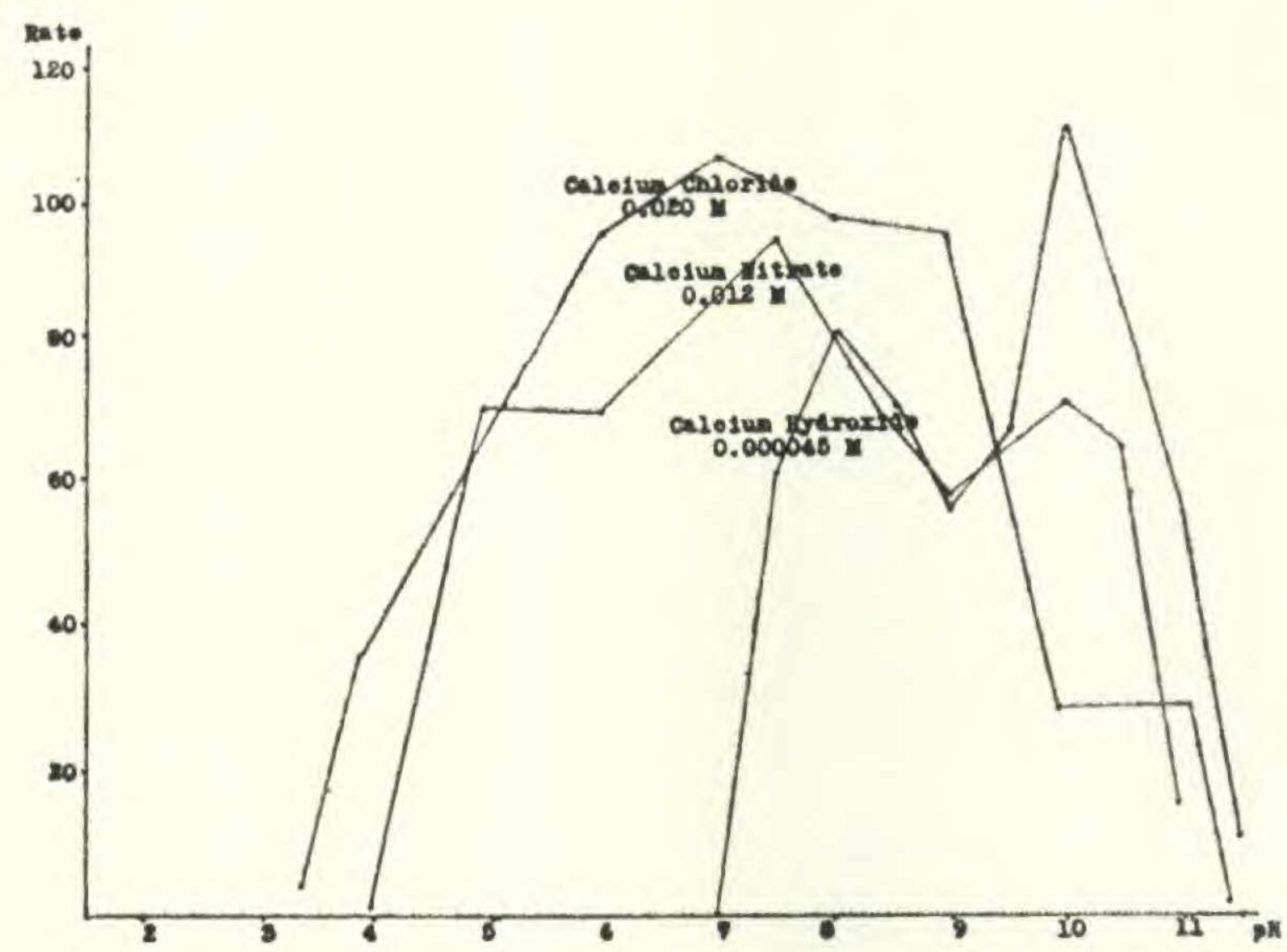

Fig. 9B. Maximum root-hair elongation in calcium hydroxide, calcium chloride, and calcium nitrate.

It is surprising to find that the maximum rate of growth occurred in a 0.000045 *M* solution of calcium hydroxide, noted earlier in No. IV of the series, while the next highest rate of growth occurred in a 0.020 *M* solution of calcium chloride. The latter solution was over 400 times as concentrated with respect to the common cation, calcium. While searching for a possible explanation upon the basis of the effects of the three anions, it became apparent that there is a possible correlation between the absolute velocities of the anions concerned and the different rates of elongation.

Since the solutions employed are exceedingly dilute, the calculations of absolute velocities may be based upon the transference numbers of the ions, the equivalent conductance at infinite dilution, and the Faraday. From the formula,

$$v = \frac{n_a \lambda_0}{F}$$

the values for the three anions are as follows:[1]

OH^{+} = 0.001802 cms. per second

Cl^{-} = 0.000676 cms. per second

NO^{-}_{3} = 0.000638 cms. per second

A comparison of these values with the rates of growth in solutions containing these anions shows a serial relationship which may be of no real significance, but which seems to be worthy of mention. The six curves for the maximum and average rates of root-hair elongation in calcium hydroxide, calcium chloride, and calcium nitrate (fig. 9) are no more striking, but represent the general tendency of the calcium chloride to support a higher rate of growth than did the calcium nitrate. The calcium hydroxide, while containing much less nutritive material and covering a much more narrow pH range, supported the highest rate of growth of all at a pH of 10.0. The fact that more dilute solutions of calcium chloride and calcium nitrate than the 0.020 *M* and 0.012 *M* respectively caused a reduction in growth rate indicates more strongly a possible effect of the anions concerned.

The studies now in progress of the growth rate of root hairs of collards in calcium sulphate may throw some additional light upon the anionic effect. If there is a real correlation between the absolute velocity of the anion in the solution and the rate of growth supported by the solution, the values obtained for calcium sulphate should fall between those for calcium hydroxide and calcium chloride.

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General Observations upon the Root

In addition to the measurement of root-hair elongation a record was kept, throughout the experiments, of the average increase in root length, the average increase in tip length, the length of the zone of aquatic root hairs upon both affluent and effluent sides of the root, the length of the interzone between the aquatic and amphibious root hairs upon both affluent and effluent sides, the

[1] Noyes, A. A., and Falk, K. G. The properties of salt solution in relation to the ionic theory. Am. Chem. Soc. Jour. **34**: p. 454. 1912.

average spacing of the root hairs, and the curvature of the roots in response to the different solutions used. A typical record sheet is shown in table III, in this instance the values obtained in a concentration of 0.060 *M* at pH concentrations of 5.0, 6.0, 7.0, 9.0, and 11.0. A summary of the records, representing in each instance the average of the numerical values obtained for six roots, is given for all of the concentrations studied in table IV. With few exceptions the increase in root length in the lower concentrations is seen to be low in the high acid and high alkaline ranges and to be highest in the region of neutrality. As the concentration of the salt increases, 0.020 *M* and 0.030 *M*, the antagonism of the calcium ion for the hydrogen ion asserts itself and the increase in root length in the acid solutions approaches or equals that in the more neutral solutions. The effect of toxic concentration of the salt has begun to assert itself in the 0.060 *M* solution to the extent that the root development is, in general, suppressed, although there remains an effect of the antagonism of the calcium ion for the hydrogen ion. The data above 0.060 *M* is indicative of the same conditions but it is not extensive enough to be compared with that from the lower concentrations.

The values for average increase in length of the hairless tip, table IV, measured at the beginning and at the end of the experiment each day, are rather irregular. The roots are seen to be well covered with hairs toward the tip in the more neutral solutions in every instance, however. The variations may be accounted for upon the basis of the many factors which are undoubtedly concerned, the toxicity of high concentrations of both the hydrogen and hydroxyl ions, the osmotic effects of concentration of the salt, the individual reactions of the roots studied, etc.

The length of the zone of root hairs shows more consistent results (table IV). The optimum neutral regions present, in every concentration which will permit comparisons with respect to this factor, the larger zones. The lengths of the zones upon the affluent and effluent sides of the root are more nearly equal in the acid and neutral solutions than in the alkaline. This indicates the degree of toxicity of the hydroxyl ion in high concentrations as shown by the delicate reaction of root-hair production.

The data for the presence and extent of an interzone or hair-

TABLE III

ROOT DEVELOPMENT IN CALCIUM NITRATE (0.060 *M*)

pH	Root No.	Root		Tip		Zone		Interzone		Space	Curv.	
		o	l	o	l	t	f	t	f		o	l
	A1	5.0	9.0	1.5	4.5	0.0	0.0	1.0	1.0	10.0	f	f
	2	2.5	6.0	1.0	2.5	1.0	1.0	1.0	1.0	4.0	s	s
5	3	3.0	5.0	1.0	2.0	0.0	0.0	0.0	0.0	10.0	s	s
	B1	2.0	4.0	1.5	3.5	0.0	0.0	0.0	0.0		s	s
	2	3.0	7.5	1.0	5.5	0.0	1.5	0.0	0.0		f	s
	3	4.5	8.0	1.0	2.5	1.5	0.0	1.5	1.5	10.0	f	s
	A1	5.0	8.5	1.5	3.0	2.0	2.0	0.0	0.0	5.0	f	s
	2	4.0	7.0	1.0	3.0	1.0	0.0	1.0	1.0	—	f	s
6	3	2.0	5.5	1.5	2.0	1.5	1.5	0.5	0.5	6.0	f	f
	B1	1.5	5.0	1.5	5.0	0.0	0.0	0.0	0.0	—	t	t
	2	2.0	6.0	1.0	2.0	1.5	1.5	1.0	1.5	5.0	s	s
	3	4.5	7.5	1.5	2.0	2.5	3.0	1.0	0.0	4.0	f	f
	A1	6.0	9.5	1.5	1.5	0.5	1.0	1.5	1.0	100.0	f	f
	2	4.0	6.0	1.5	2.0	1.5	1.5	0.0	1.0	2.0	t	t
7	3	2.5	5.5	1.5	2.0	1.5	2.0	1.0	0.0	4.0	f	s
	B1	2.0	5.5	1.5	2.0	3.0	3.0	0.0	0.0	8.0	t	s
	2	3.0	6.5	1.0	2.0	2.5	2.5	0.0	1.0	7.0	f	s
	3	5.5	8.0	1.5	3.0	0.3	0.0	0.7	1.0	10.0	t	s
	A1	6.0	7.5	1.5	1.5	1.0	1.0	0.5	0.5	1.0	t	s
	2	3.5	6.0	1.0	3.0	1.0	1.0	0.0	0.0	1.0	s	s
9	3	2.0	5.5	1.0	3.0	1.5	1.5	0.0	0.0	6.0	s	s
	B1	1.5	4.0	1.0	3.0	0.5	0.5	0.0	0.0	1.0	s	s
	2	2.0	6.0	1.5	3.0	2.5	2.5	0.0	0.0	5.0	s	s
	3	6.0	10.0	2.0	2.0	2.5	2.5	1.0	1.0	8.0	t	s
	A1	5.0	7.0	2.0	2.0	1.0	1.0	1.0	1.0	2.0	s	s
	2	3.0	5.5	2.0	2.5	1.0	1.0	1.0	1.0	4.0	t	s
11	3	1.5	3.5	1.0	2.5	0.0	1.0	1.0	0.5	3.0	t	s
	B1	1.5	4.0	1.0	3.0	0.0	0.5	0.5	0.0	5.0	t	s
	2	3.0	5.0	1.5	2.0	0.25	0.5	0.5	0.5	5.0	s	s
	3	4.0	5.5	2.0	2.0	0.5	1.5	1.0	1.0	3.0	s	s

Values for Root, Tip, Zone, and Interzone in terms of millimeters (1 = 3.6 mm.); values for Space in terms of microns (1 = 3.3 μ).

Zone = region of aquatic root hairs.

Interzone = hairless space between amphibious and aquatic root hairs.

Space = approximate average distance between adjacent root hairs on the horizon of the root, in microns.

Curvature = direction of curvature of the root, s, straight, t, affluent, f, effluent.

A = first chamber.
B = second chamber.
o = original length.
l = final length.
t = affluent side of the root.
f = effluent side of the root.

TABLE IV

A SUMMARY OF THE ROOT DEVELOPMENT IN CALCIUM NITRATE

Conc. *M*	pH	Root			Tip			Zone		Interzone		Curv.		
		o	l	d	o	l	d	t	f	t	f	s	t	o
	5.0	4.0	7.6	3.6	1.6	2.1	0.5	3.0		0.0		4	1	1
	6.0	4.3	8.0	3.7	1.6	2.2	0.6	3.0		0.0		4	2	0
	7.0	3.0	7.3	4.3	1.2	2.2	1.0	3.3		0.2		4	1	1
	7.5	4.6	8.7	4.1	1.3	2.0	0.7	3.7		0.0		6	0	0
0.004	8.0	3.4	8.1	4.7	1.2	2.4	1.2	3.5		0.0		6	0	0
	9.0	3.9	7.6	3.7	1.4	2.4	1.0	2.5		0.2		6	0	0
	10.0	4.0	8.8	4.8	1.4	2.2	0.8	3.0		0.2		3	3	0
	11.0	4.0	6.4	2.4	1.5	1.8	0.3	2.0		0.2		1	4	1
	11.5	3.4	4.6	1.2	1.4	3.4	1.0	0.0		—		0	5	1
	4.5	3.6	6.8	3.2	1.4	2.2	0.8	0.8		0.6		4	1	1
	6.0	4.3	8.3	4.0	1.6	2.0	0.4	3.5		0.2		5	0	1
	7.0	4.3	8.1	3.8	1.5	2.1	0.6	3.2		0.3		4	1	1
0.008	8.0	4.5	8.8	4.3	1.5	2.0	0.5	3.5		0.5		4	1	1
	9.0	3.3	7.5	4.2	1.5	2.0	3.0	1.0		0.6		4	2	0
	10.0	4.0	6.6	2.6	1.0	2.0	1.0	2.1		0.6		0	6	0
	10.5	3.6	8.6	5.0	1.1	2.0	0.9	2.0		0.0		0	6	0
	4.0	2.3	4.1	1.8	1.1	2.1	1.0	—		—				
	5.0	3.2	6.4	3.2	1.3	2.4	1.1	2.9	2.9	0.08	0.16	3	1	2
	6.0	4.1	8.6	4.5	1.6	2.1	0.5	3.8	3.8	0.13	0.13	6	0	0
	7.0	4.2	10.4	6.2	1.5	2.4	0.9	4.4	4.6	0.10	0.6	6	0	0
0.012	8.0	3.0	6.2	3.2	1.08	2.1	1.02	2.1	2.2	0.0	0.1	6	0	0
	9.0	4.5	7.6	3.1	1.5	1.9	0.4	1.6	1.2	0.5	0.55	4	2	0
	10.0	3.1	6.6	3.5	1.1	1.8	0.7	2.2		0.2		1	5	0
	10.5	4.4	7.6	3.2	1.5	1.7	0.2	2.7		0.6		0	6	0
	11.0	4.1	6.5	2.4	1.4	1.6	0.2	1.08	0.54	0.5	1.08	3	3	0
	4.5	3.6	7.6	4.0	1.8	3.3	1.5	0.76	0.76	1.1	1.0	5	0	1
	6.0	4.1	7.6	3.5	1.5	2.6	1.1	2.6	2.6	0.3	0.3			
	7.0	3.5	8.3	4.8	0.7	1.6	0.9	3.0	2.8	0.0	0.13	6	0	0
0.020	8.0	4.0	7.8	3.8	1.6	2.2	0.6	3.98	3.13	0.4	0.2	5	0	1
	9.0	3.8	6.5	2.7	1.3	2.08	0.78	1.3	2.2	0.0	0.0	2	2	0
	10.0	3.8	6.8	3.0	1.6	2.1	0.5	2.3	3.0	0.2	0.0	6	0	0
	11.0	3.25	5.6	2.3	1.1	1.0	0.1	0.35	1.1	1.3	0.5	2	0	4
	5.0	3.6	7.6	4.0	1.6	2.5	0.9	0.4	2.0	1.4	1.4	6	0	0
	6.0	2.2	6.4	4.2	1.1	2.8	1.7	2.2	2.2	0.0	0.0	6	0	0
	7.0	3.7	7.4	3.7	2.8	2.7	−0.1	2.6	2.7	0.3	0.4	2	3	1
	8.0	4.1	7.2	3.1	1.2	2.6	1.4	2.0	2.0	0.13	0.1	4	1	1
0.030	9.0	3.5	6.4	2.9	1.3	2.4	1.1	1.3	1.7	0.1	0.15	6	0	0
	9.5	3.6	5.7	2.1	1.0	1.8	0.8	0.8	1.6	3.5	1.5	5	1	0
	10.5	2.6	4.8	2.2	1.1	2.0	0.9	0.6	1.1	0.4	0.3	6	0	0
	11.0	2.6	5.4	2.8	1.1	1.7	0.6	0.2	1.8	1.0	0.3	1	0	5
	11.5	2.6	5.4	2.8	1.3	1.4	0.1	0.0	0.3	0.5	0.5	2	3	1

TABLE IV—*Continued*

Conc. M	pH	Root			Tip			Zone		Interzone		Curv.		
		o	l	d	o	l	d	t	f	t	f	s	t	o
0.060	5.0	3.3	6.6	3.3	1.1	3.8	2.7	0.25	0.25	0.58	0.58	5	0	1
	6.0	3.1	6.6	3.5	1.3	2.8	1.5	1.2	1.3	0.58	0.5	3	1	2
	7.0	3.8	6.8	3.0	1.4	2.1	0.7	1.55	1.66	0.53	0.5	4	1	1
	9.0	3.5	6.5	3.0	1.3	2.6	1.3	1.5	1.5	0.25	0.25	6	0	0
	11.0	3.0	5.1	2.0	1.6	2.3	0.7	0.46	0.91	0.83	0.66	0	6	0
0.100	5.0	2.2	3.1	0.9	1.2	1.3	0.1	0.6	0.6	0.5	0.5	5	1	0
	8.0	4.2	5.5	1.3	1.3	1.75	0.45	0.62	2.0	0.50	0.54	5	0	1
	10.5	4.0	5.5	1.5	1.3	1.2	−0.1	0.46	0.54	0.6	0.6	5	1	0
0.120	7.0	3.6	4.6	1.0	1.4	1.3	−0.1	0.8	0.8	0.5	0.3	6	0	0
0.140	7.0	3.5	4.0	0.5	1.0	0.8	−0.2	0.5	0.5	0.3	0.3	6	0	0

Values for Root, Tip, Zone, and Interzone in terms of mm. (1 = 3.6 mm.).
o = original length.
l = final length.
d = l − o.
t = affluent side of the root.
f = effluent side of the root.
s = straight.
o = effluent curvature.
t = affluent curvature.

less region between the portions of the root covered by amphibious hairs and aquatic hairs will not, in every instance, support the author's explanation of the findings in calcium hydroxide (paper VII.) The interzone in these instances was believed to have been produced by an extremely rapid growth of the root, so rapid that the production of root hairs in the horizontal direction was, for a time, suppressed. In calcium nitrate solutions, however, the highest rates of increase in root length do not always correlate with the greatest extent of interzone. The results would indicate that other factors may be involved in this very conspicuous reaction. The findings in calcium nitrate with respect to the interzone would tend to support more strongly the general conclusion in paper V, in which the author explained the presence of the interzone through the temporary suppression of root-hair development, and not as a result of more rapid root elongation.

The assumption that the normal direction of root growth is straight, and that curvatures in either the affluent or the effluent direction represent reactions to toxic conditions, is well substantiated (table IV). In the neutral solutions of lower concentration the six roots, or a large percentage of them, remained straight in most cases. Here again the high concentrations of hydrogen ions seemed to be less toxic than the excessive amounts of hydroxyl ions, a smaller number of roots having shown curvature in the more acid than in the more alkaline solutions. The antagonism of the calcium ion for the hydrogen ion comes out again in solutions of 0.020 *M* and above, the roots showing little or no curvature in the most acid solutions studied in these concentrations.

It may be seen from the data in table IV that interzone formation in 0.012 *M* $Ca(NO)_3$ solutions is more extensive on the effluent side, while in 0.020 *M*, 0.030 *M*, and 0.060 *M* solutions it is more extensive on the affluent side. This reaction would seem to be a response to the degree of acidity or alkalinity of the solutions rather than to their salt concentrations. When the data for interzone formation is presented in terms of salt concentration, this tendency to greater evidence of toxicity upon the affluent side seems to be less conspicuous.

The extent of the zone of root hairs in 0.012 *M* solutions of different H-ion concentrations shows little difference upon the affluent and effluent sides. In 0.020 *M*, 0.030 *M*, and 0.060 *M* solutions, the greater length of zone is found upon the effluent side. These results confirm, in general, the author's earlier suggestion, that in toxic solutions a higher degree of injury was registered upon the affluent side in the form of the presence of an interzone, while a lower degree of toxicity is shown in the more abundant production of root hairs upon the effluent side.

A more general view of the relationship between the concentration of the salt and the various factors presented in table IV may be obtained through a further condensation of this data (table V). Every value here represents the average of the average values obtained in the hydrogen-ion concentrations of the solutions studied. A comparison of these values for root elon-

TABLE V

SUMMARIZATION OF THE DATA GIVEN IN TABLE IV, TO BRING OUT THE EFFECT OF CONCENTRATION OF THE SALT

Conc. *M*	Root length	Tip length	Zone		Interzone		Space
			t	f	t	f	
0.004	3.6	0.8	2.7		0.1		5.8
0.008	7.5	1.0	2.3		0.40		5.9
0.012	3.4	0.67	2.64	2.5	0.26	0.42	5.5
0.020	3.4	0.78	2.04	2.2	0.47	0.30	6.2
0.030	3.09	0.92	1.1	1.7	0.80	0.50	6.8
0.060	2.9	1.4	1.09	1.1	0.5	0.60	4.4
0.10	1.2	0.15	0.56	1.04	0.50	0.50	2.3
0.120	1.0	−0.1	0.80	0.80	0.50	0.30	2.0
0.140	0.50	−0.2	0.50	0.50	0.30	0.30	1.0

gation with those for root-hair elongation in different concentrations of calcium nitrate (fig. 2, paper II), brings out again the tendency to a more delicate reaction by the root hairs than is shown by the root as a whole. The optimum concentration for root elongation, 0.008 *M*, falls near to the optimum for root-hair elongation, 0.012 *M*. The differentiation upon either side of these optima is greater, however, in the root hair than in the root. The data for tip length show that the largest total amount of surface was covered by hairs at a concentration of 0.012 *M*, a point well within the range of solutions which maintained the best conditions for growth of root hairs as well as roots. The values in 0.120 and 0.140 *M* again represent such a limited amount of data that they may be omitted in this comparison. The highest value for the extent of the zone of root hairs occurs at 0.004 *M*, is maintained near to this point in 0.008 *M* and 0.012 *M*, and then declines gradually with a slight increase at 0.120 *M*. The extent of the interzone shows a greater tendency to fluctuate with change in concentration. It is of especial interest to observe the apparent lack of effect of concentration of the salt with respect to unequal growth of root hairs upon the affluent and effluent sides of the root, as shown by the data from the zones and interzones. The values upon the two sides show very little difference, and these differences are not consistent with the findings in solutions of toxic hydrogen- and hydroxyl-ion concentrations reported earlier by the author. It will be remembered that the greater effect of toxicity in all of the latter type of solutions studied appeared upon the affluent side. The spacing of root hairs is also quite constant in all concentrations which will permit comparison. The values in this connection would undoubtedly be much higher in concentrations from 0.10 *M* to 0.140 *M* if the experiments had covered a range of solution of varying degrees of acidity and alkalinity comparable to that in lower concentrations.

The marked effect of hydrogen-ion concentration of nutrient media upon a wide variety of organisms continues to appear from many sources. Herčik (205) has recently reported a series of experiments upon root elongation in *Pharbitis*, with methods similar to those used in the present study. Weak buffer solutions of primary and secondary sodium phosphate (M/100) were allowed to flow over the roots. To increase the acid N/10 H_2PO_4 was added, and to increase the alkali N/10 tertiary sodium phosphate was added. Electro thermal regulation at 20° C. was maintained in a darkened under-ground room. All of the material for study was prepared in the same place under the same conditions of light, humidity, and temperature. The seedlings were grown in solutions of a definite hydrogen-ion concentration and then transferred suddenly to solutions of differing degrees of acidity. The change in rate of growth was noted every 3 to 5 minutes for 25 minutes. It is interesting to observe the similarity of the curve of permanent growth so obtained to the curve which was shown by the author for collards in a solution of 0.060 *M* calcium chloride, the acid and the alkaline optima, in both instances, falling at 5.8 and 7.6 respectively with the median minimum at 6.2.

Pantin (211), studying the growth of Amoeba in hay infusions ranging in pH values from 5 to 9, found that they thrive best at a pH of 8.2. A bimodal curve was obtained with a median minimum at a pH of 7. Taylor states that amoebae live between pH 3 and pH 8, but thrive best at pH 6.6. Hopkins (206), in a later paper, has given a fuller treatment of the effect of hydrogen-ion concentration upon growth and reproduction in Amoeba. Marked reactions to slight changes in pH were found.

At a pH of 7.2 all were dark and spherical. At a pH of 7.4 nearly all were again normal in appearance, moving about and feeding actively, and at a pH of 7.6 they were larger and more numerous. When the hydrogen-ion concentration had dropped to pH 7.8 the amoebae again became dark and sluggish, but not spherical. Further study of the relation between behavior and hydrogen-ion concentration resulted in a bimodal curve with acid and alkaline optima at pH 6.6 and 8.0–7.6, with a median minimum at about pH 7.1. In explanation of the phenomena the author relies upon the idea of changes in permeability of the membranes due to different hydrogen- and hydroxyl-ion concentrations of the solutions. With the increase in permeability to the salts, an increase in the internal osmotic pressure takes place, and consequently an increase in the water content of the amoeba and a decrease in the rate of locomotion.

Recent studies by Loo (209) on the effect of different pH concentrations upon the growth of seedlings further substantiate the idea of the antagonism of calcium ions for hydrogen ions. His experiments extended over an 8-day period, the seedlings having been introduced into the culture solution when the shoots were 3–9 cms. long. In the presence of calcium ions wheat grew fairly well in a pH of 3 or 4. In the absence of calcium this degree of acidity retarded the growth. Kaho (207), in a study of the roots of legumes, has found that K, Na, NH_3, and Mg injury in a short time begins to kill the roots. The root tip becomes slimy, and the growing zone becomes glassy and transparent. This is accompanied by a cessation in growth of the stem and a drying-up of the heads. The addition of calcium, however, inhibits most of these toxic effects.

A discussion of the necessity for the presence of calcium in connection with the development of many organisms was taken up in an earlier paper of the series (I). It is quite generally believed that its absence leads to the destruction of the cell. It has been considered to be an important component of chloroplasts, nuclei, and membranes in the form of compounds of plastin, nuclein, calcium pectate, callose, etc. Hansteen-Cranner (203), studying the effects of pure salts, found that the cause of toxicity of the salt is not in the structural change of the interior of the cell but in the surface effect. The amount of calcium needed to counteract injurious effects of various salts varies greatly. The quantity needed to overcome the effect of the magnesium ion was far in excess of that needed to antagonize the potassium ion. Sokoloff (201), in a discussion of the dying and super-active (neoplastic) cells to be found in cancer tissue, attributes the rejuvenation in the latter, as shown by their nucleo-plasma ratio, mitochondria, glycogen content, etc., to an alteration of the cellular membrane and cellular lipoids. Nuclear changes, he considers to be secondary in producing the unusual behavior and mentions the possibility that the pathogenic elements may be outside the cell in the lymphoid elements. An interesting study of the relation of calcium to the plasma membrane of eggs of *Arbacea* and *Stentor* has been made recently by Heilbrunn (204). When the eggs were crushed it was observed that, in some instances, films were formed about the extruded cytoplasm, while in others there were no films produced. A series of experiments which followed demonstrated that the film was not due to adsorbed lipoids; that calcium was necessary for the reaction; that magnesium and barium could not be substituted for calcium; that the membrane formed in the absence of calcium is due to the presence of "Ovothrombin"; that the ovothrombin is probably made up of calcium and pigment granules; that the calcium may be in loose combination with a lipoid substance; and that it is freed when the lipoid substance breaks down.

The accumulation of larger amounts of data dealing with simple ionic effects upon various organisms will undoubtedly lead to a clearer understanding of the real nature of the factors concerned in growth. With respect to the rate of root-hair elongation and the other points of interest in root development which have been discussed there has been found to be little difference in the roots of collards grown in calcium nitrate and those grown in calcium hydroxide and chloride. The morphological changes in root hairs have shown greater contrasts in the different salt solutions used and have been treated in a separate paper (VIII).

Moravek (210) has attempted to gain a clearer understanding of the growth conditions in root hairs by observing the growth of structures formed by the reaction on the boundary between solutions of electrolytes in water and those in a gel. Upon a solidified 0.1 N solution of potassium dichromate in 5 per cent gelatin was placed a layer of 0.1 N solution of lead nitrate. Fibrous structures 0.1 to 0.3 mm. wide grew out into the latter. Their walls were formed by a gelatin membrane with precipitated lead chromate. A stream proceeded through the fiber from the gelatin layer, meeting the lead nitrate solution at the tip of the fiber. A precipitated gelatin layer in the form of a membrane upon which lead chromate is deposited discontinuously grew with a maximal velocity of 0.16 mm. per minute. The chief growth directions are vertical and horizontal. The growth velocity and the dimensions and the shape of the fibers were influenced by the concentration of the gelatin, the addition cf calcium or potassium ions, and the raising of the temperature above 32° C. Light intensity seemed to exert no influence. The cause of the growth in the fiber was attributed to the diffusion stream. This conclusion is entirely in keeping with the findings and interpretations of the author in connection with the diffusion streams in root hairs of collards and discussed in another paper dealing with morphological changes in root hairs of collards on solutions of calcium nitrate (VIII). From observations upon the development of twin hairs, in which the movement of the nucleus into one branch of the hair was accompanied by cessation of growth in that branch, the idea was advanced that the retardation had been caused by the blocking of the diffusion stream by the nucleus.

Conclusions

1. Improvements in the methods of determination of the hydrogen-ion content of the solutions used are described.

2. The curves for the rate of root-hair elongation of collards in dilute solutions of calcium nitrate are shown to be trimodal in contrast to the bimodal curves obtained in calcium chloride.

3. The curves for the rate of root-hair elongation in median concentrations of calcium nitrate are seen to be bimodal, as are those obtained in all except the most concentrated solutions of calcium chloride.

4. The curves for the rate of root-hair elongation in solutions of higher concentrations are monomodal, as were the curves in high concentrations of calcium chloride.

5. The three-dimensional graph from solutions in calcium nitrate shows in dilute solutions, therefore, three optima and two median minima, or perhaps the latter should be called the acid minimum and the alkaline minimum.

6. The median or neutral optimum is shown to be slightly zig-zag. It may be assumed, however, that if the data had been obtained at pH 7.5 instead of at pH 7.0 and 8.0 in the median concentrations that this would have been a straight line also.

7. A floor-plan of the three-dimensional graph for calcium nitrate bears comparable relationships to the limits that were found in calcium chloride.

8. The comparison which may be made by superimposing the one floor-plan upon the other shows that the plant will produce root hairs in a more alkaline solution in nitrate than in chloride.

9. In weak acid solutions nitrate will support root-hair growth better than will chloride.

10. In moderate concentrations root hairs will grow in a more acid solution in chloride than in nitrate.

11. It may be seen that the optima for the two salts bear a definite relation to each other, and that by the shifting of them in the nitrate toward the alkaline side, and the pushing of the acid limit to the lower acid concentrations, room is developed for the insertion of a new acid optimum.

12. The curves obtained for maximum rate of root-hair elongation, for root elongation, and for maximum length of root hairs correspond quite closely to those for average rate of root-hair elongation.

13. In 0.004 *M* solutions root elongation gives a tri-modal curve such as we obtain in root-hair elongation. The locations of the modes do not, however, correspond except in the case of the alkaline optimum.

14. In 0.012 *M* solutions the graphs for the four factors are almost identical, except that the root does not give as good differentiation upon the alkaline side as do the other factors.

15. In solutions of 0.020 *M* there is again a close similarity

in the four graphs. In this instance, however, the acid optimum for the root is at pH 6.0 instead of at pH 8.0 as in the root-hair graphs.

16. In the curves for 0.030 *M* there is a correspondence of root and root-hair activity, but here again the acid optimum for the root is at pH 5.9 instead of at 6.9, as in the root hairs.

17. Curves for 0.060 *M* solutions show a similar correspondence of root and root-hair activity except that, in this case, the acid optimum for the root is at 5.4, instead of at 4.4 for the root hair.

18. It thus appears that there is less variation of the acid optimum with change in concentration of the salt for the root than for the root hair. This may be correlated with the failure of the external solution to impinge its full effect upon the cells on the interior of the root.

19. A summarization of the entire mass of data obtained with reference to the increase in root length, the increase in tip length, the length of the zone of root hairs, the length of the interzone between the amphibious and aquatic hairs, and the spacing of the root hairs is made to bring out the effect of hydrogen-ion concentration in each of the salt concentrations used.

20. A further condensation of this data, so that each concentration at all of the points of acidity and alkalinity will be represented by one value, brings out the effect of salt concentration upon the various factors.

21. We have by this method a means of comparing different chemical ions as to their biological effect on a more or less accurate mathematical basis.

22. It is believed also that the method presents an accurate method of study of the specific effect of different substances upon a simple cell process, namely, cell enlargement.

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HYSTERANGIUM IN NORTH AMERICA[1]

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HYSTERANGIUM

Hysterangium Vittadini, Monogr. Tuberac. 13–15. 1831; Tulasne, Fung. Hypog. 80–85. 1851; Winter in Rabenhorst, Krypt.-Fl. Deutschl. ed. 2, I. **1**: 878–879. 1883; DeToni in Sacc. Syll. Fung. **7**: 155–158. 1888; Hesse, Hypog. Deutschl. **1**: 94–105. 1891; Harkness, Cal. Acad. Sci. Proc. III. **1**: 254–257. 1899; E. Fischer in Engl. & Prantl, Die Nat. Pflanzenfam. I. **1****: 306. 1899; Bucholtz, Материалы къ морфологіи и систематикѣ подземныхъ грибовъ (Tuberaceae И Gastromycetes pr. p.) съ приложеніемъ описанія видовъ, найденныхъ до сихъ поръ въ предѣлахъ Россіи. Издан. Естеств. Ист. Музея Графини Е. П. Шереметевой въ с. Михайловскомъ Московской Губ. **1**: 151–153. 1902 [sometimes cited as Beitr. Morph. Syst. Hypog.]; Th. M. Fries, Svensk Bot. Tidskr. **3**: 279–281. 1909; Rodway, Roy. Soc. Tasmania Papers & Proc. **1911**: 26–27. 1912; Soehner, Pilz und Krauterfreund **5**: 254–256. 1922.

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The type species of the genus is considered to be *Hysterangium clathroides* Vittadini in accordance with the recommendations of the Committee on Nomenclature of the Botanical Society of America, Bot. Soc. Am. Publ. 73: 70–71. 1919, since the author states that he has collected this species many times and describes it at greater length.

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